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DNA Polymorphism in Morels: Complete Sequences of the Internal Transcribed Spacer of Genes Coding for rRNA in *Morchella esculenta* (Yellow Morel) and *Morchella conica* (Black Morel)

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The internal transcribed spacer (ITS) of the gene coding for rRNA was sequenced in both directions with the gene walking technique in a black morel (*Morchella conica*) and a yellow morel (*M. esculenta*) to elucidate the ITS length discrepancy between the two species groups (750-bp ITS in black morels and 1,150-bp ITS in yellow morels).

In members of the family Morchellaceae, isozymes (10, 16, 19, 25, 27) and the gene coding for the 28S rRNA (rDNA) (4, 5) sometimes exhibit higher intraspecific than interspecific variations, illustrating the difficulty of systematics and the species concept in the family (2, 3, 9, 15). Recently, Buscot et al. (6) showed that polymorphism of the internal transcribed spacer (ITS) of rDNA is more adequate to improve morel systematics. Both species groups recognized in all classifications, i.e., black (*sectio distantes*) (14, 21) and yellow (*sectio adnatae*) morels, exhibited respective ITS lengths of 740 to 750 and 1,150 to 1,220 bp (6). Furthermore, slight length variations and different restriction profiles allowed the identification of several species within each group (26). In the present work, the whole ITS region of a black morel (*Morchella conica*) and a yellow morel (*M. esculenta*) was sequenced to identify the origin of the ITS length difference between the two species groups.

The analyses were performed on monosporal isolates of *M. esculenta* and *M. conica*, which are deposited under the accession numbers DSM 10374 and DSM 10464 in the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The strain cultivation, DNA extraction, and ITS PCR methods used were similar to those described by Buscot et al. (6); the ITS1 and ITS4 primers were used to amplify the ITS region plus a small portion each of the 3′ end of the 17S rDNA and the 5′ end of the 25S rDNA (24). The amplified DNAs were purified with microspin columns (Pharmacia) and sequenced in both directions by Genome Express (Grenoble, France). The sequencing reaction was performed by PCR amplification in a final volume of 20 ml using 100 ng of PCR products, 5 pmol of primers, and 9.5 ml of premixed Dye Terminators in accordance with the Applied Biosystems protocol. After heating to 94°C for 2 min, the reaction mixture was subjected to 25 cycles of 30s at 94°C, 30s at 55°C, and 4 min at 60°C (9600 thermal cycler; Perkin Elmer). Removal of any excess of Dye Terminators was performed with Quick Spin columns (Boehringer Mannheim). The samples were dried in a vacuum centrifuge and dissolved with 4 ml of deionized formamide-EDTA (5/1 ratio) at pH 8.0. The samples were loaded onto an Applied Biosystems 373A sequencer and run for 12 h on a 6% denaturing acrylamide gel. Sequence data were stored, assembled, and analyzed with the biosequence editor and analysis application SeqApp version 1.9 (12) on an Apple Macintosh II SI computer. Multiple alignment of sequences was performed with the Clustal V package (13) interfaced to SeqApp. Nucleotide sequence comparisons were also performed at the National Center for Biotechnology Information by using the Basic Local Alignment Search Tool network service (1) against the National Center for Biotechnology Information databases (Non-redundant PDB, GBupdate, GenBank, EMBLupdate, and EMBL; 1 March 1996). The whole procedure (cultivation, DNA extraction, ITS amplification, and sequencing) was performed twice for both species.

All amplification products were unique fragments with respective lengths of 1,133 (*M. esculenta*) and 710 (*M. conica*) bases that were identical in all replicates. In both fungi, one sequence extremity was similar to the 3′ end of the ITS4 primer over 16 bases (total length of the primer, 20 bases). For *M. esculenta*, the other sequence extremity was highly complementary (18 of 19 bases) to the ITS1 primer; the base inversion was obviously due to a sequencing error. In the *M. conica* sequence, the ITS1 primer was totally lacking, indicating sequencing interruption. However, sequence alignment with *M. esculenta* indicated that probably only five bases of the *M. conica* ITS were not sequenced at this end. Because of the length of the analyzed region, sequencing without intermediary primers was delicate and led to these minute imprecisions or interruptions at the ends of the sequences. However, these did not prevent a clear interpretation. Furthermore, Basic Local Alignment Search Tool comparison revealed high-scoring seg-

TABLE 1. Comparison of the positions, lengths, and homologies of three regions in the ITS of the rDNAs of *M. esculenta* and *M. conica*

DNA	Localization, length (bp) of ITS region		% Homology between *M. esculenta* and *M. conica*
	M. esculenta	*M. conica*	
ITS1 spacer	44–538, 494	16–212,197	42.13
5.8S gene	539–711, 173	213–385,173	97.7
ITS2 spacer	712–1080,369	386–657,272	35.29

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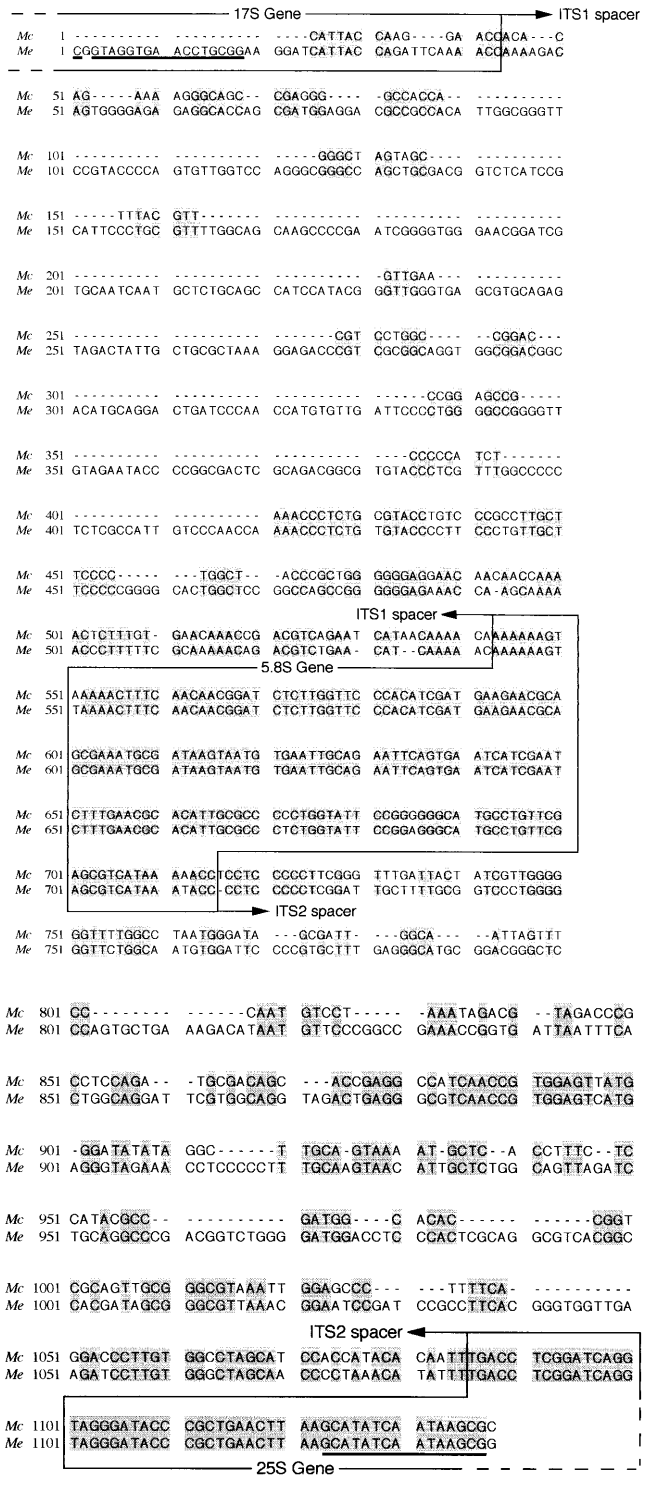

FIG. 1. Sequence alignment of the ITS of the rDNAs of *M. esculenta* (*Me*) and *M. conica* (*Mc*). Underlined bases indicate complementarity with the primers used to amplify the target region.

ment pairs (over 83%) exclusively between limited sectors of the sequences (bases 519 to 742 in *M. esculenta* and bases 163 to 404 in *M. conica*) and the 5.8S rDNAs of other ascomycetes. This confirmed that the amplified and sequenced regions both correspond to the morel ITS.

The high homologies with the 5.8S rDNAs of other ascomycetes allowed localization of the 5.8S gene and of the ITS1 and ITS2 spacers in the morels (Table 1 and Fig. 1). High sequence homology was observed between the 5.8S genes of both morels (Table 1). In contrast, the noncoding spacers ITS1 and ITS2 exhibited polymorphisms (Table 1 and Fig. 1). Good homologies characterized both the extremities of the spacers bordering the 5.8S gene and the 3′ end of ITS2 (Fig. 1). Length discrepancy in ITS2 corresponded to several small additional fragments (up to 15 bases) between bases 793 and 1046 of the *M. esculenta* ITS (Fig. 1). Length discrepancy between the ITS1 spacers of both morels was concentrated principally between bases 89 and 420 of the *M. esculenta* ITS (Fig. 1). This region with insertions (40 to 68 bases) in *M. esculenta* showed only 25.1% homology between the two morels.

At the genus level, the degree of polymorphism in the noncoding ITS1 region varies among fungi (20). Less than 2% divergence and a constant length (200 bp) characterize the ITS1 regions of different *Sclerotium* species (7), whereas *Colletotrichum* species exhibit a homogeneous length (170 bp) with up to 16.5% base substitutions (22, 23). Previous reports have mentioned a constant length of the entire ITS at the genus level, with the polymorphism between species reduced to base substitutions of up to 16% (8, 17, 18). The length of the entire ITS diverges dramatically between *M. esculenta* and *M. conica*—a similar situation was described in the genus *Tuber*, another higher discomycete (11)—because of insertion-deletion substitutions in both the ITS1 and ITS2 spacers, interspersed with small conserved regions.

Nucleotide sequence accession numbers. The GenBank accession numbers for *M. esculenta* A7 and *M. conica* Co1 are U51851 and U51852, respectively.

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